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FOR DATE(S) _____
BY ES _____

12 UNITED STATES DISTRICT COURT
13 NORTHERN DISTRICT OF CALIFORNIA
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12 CHIRON CORPORATION,
13 Plaintiff,
14 v.
15 ABBOTT LABORATORIES,
16 Defendant.
17

C 93 4380 MHP

**EXPERT REPORT OF B. MATIJA
PETERLIN, M.D. PURSUANT TO
FEDERAL RULE OF CIVIL
PROCEDURE 26**

18 I. QUALIFICATIONS

19 I am a Professor of Medicine, Microbiology and Immunology, at the
20 University of California, San Francisco, and an Investigator, Howard Hughes Medical
21 Institute at UCSF. My primary area of research has been focussed for the past ten
22 years on the study of the molecular biology of HIV. A copy of my curriculum vitae is
23 attached hereto as Exhibit A.
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EXPERT REPORT OF B. MATIJA PETERLIN, M.D.
PURSUANT TO FEDERAL RULE OF CIVIL PROCEDURE 26

1 II. OPINIONS

2 A. Chiron's expression from the EcoRI-KpnI fragment from the
3 ARV2 HIV¹ isolate resulted in an immunoreactive Env polypeptide.

4 Chiron's patent application No. 867,501 describes the identification,
5 cloning and expression of the EcoRI-KpnI fragment from ARV2 in COS cells. We
6 now know that this fragment contains open reading frames for Tat, Rev, Vpu, and Env
7 (Gallo 1988). Staining with an AIDS antiserum revealed cytoplasmic fluorescence only
8 in transfected cells ('501 Application, Sanchez-Pescador 1985). Subsequent reports by
9 many investigators confirmed that this cytoplasmic staining was due to the expression
10 of Env. These investigators also identified Env by electrophoretic mobility and
11 immunoreactivity testing of lysates from these cells (Rekosh 1988, Bird 1990, Kimura
12 1994).

13 Schwartz et al., are the only group which did not detect the expression of
14 Env with a fragment similar to the EcoRI-KpnI fragment from ARV2. In a HeLa cell
15 expression system, they detected only Tat (Schwartz 1990). However, they expressed
16 Env from the same fragment in the rabbit reticulocyte lysate, which is a different
17 expression system. Schwartz et al. acknowledged that other investigators expressed
18 Env with such fragments and were puzzled by their inability to do the same in HeLa
19 cells.

20 Given the data in the Chiron patent and subsequent experiments from
21 many groups, an ordinarily skilled scientist in 1984 or today could conclude confidently
22 that the EcoRI-KpnI fragment from ARV2 expressed Env in COS cells.

23

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25 1 The virus that causes AIDS is now known as HIV, or human immunodeficiency virus. In
26 1984, when it was first isolated and characterized, it had several names given it by various
27 research groups: LAV (by Dr. Luc Montagnier's group at the French Institut Pasteur); HTLV-
28 III (by Dr. Robert Gallo's group at the National Institutes of Health); and ARV (by Chiron's
collaborator Dr. Jay Levy at the University of California, San Francisco). The name "HIV"
was established in 1986, and is used throughout this report for convenience (Coffin 1986).

1 B. An ordinarily skilled scientist in October 1984 could conclude that
2 the peptide expressed from the EcoRI-KpnI fragment of ARV2 was from Env.

3 In October 1984, Tat, Rev, and Vpu were not known (Sanchez-Pescador
4 1985, Ratner 1985, Wain-Hobson 1985, Muesing 1985). An ordinarily skilled scientist
5 following the example set out in the Chiron application would have expected to express
6 Env and would have done so. By the intense cytoplasmic staining of transfected COS
7 cells which is consistent with the presence of Env, the immunofluorescence test
8 described in the application would have confirmed the expression of Env (Rekosh
9 1988, Bird 1990, Kimura 1994). Tat and Rev proteins have been localized to the
10 nucleus and do not stain in the cytoplasm (Hauber 1987, Ruben 1989, Siomi 1990,
11 Cullen 1988, Felber 1989, Hope 1990). Nuclear staining is easily distinguished from
12 cytoplasmic staining in COS cells, which adhere to and spread out on glass or plastic
13 support (Gluzman 1981). Once the immunofluorescence had confirmed Env, the
14 product of the expression system could have been used by an ordinarily skilled scientist
15 as a recombinant Env immunoassay in 1984. The presence (unknown at the time) of
16 Tat, Rev, and Vpu along with Env would not have impeded the effectiveness of the
17 binding of Env with an AIDS antiserum.

18 In the case of the ARV2, the cytoplasmic staining was due to the
19 expression of Env alone. Vpu is an integral membrane protein, which contains 81
20 amino acids and measures 16 kDa (Cohen 1988, Strebel 1988, Maldarelli 1993).
21 However, not only do many viral strains contain stop codons in *vpu*, but the ARV2
22 sequence as disclosed in the Chiron application contains a stop codon at position 38 in
23 *vpu* (Sanchez-Pescador 1985, Cohen 1988, Myers 1994). Since all reported
24 immunoreactive epitopes of Vpu are encoded by sequences beyond this stop codon
25 (Schneider 1990, Kusk 1993), they would not be included in the protein expressed, and
26 an AIDS serum would not recognize this truncated Vpu peptide from ARV2. Thus,

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1 Chiron scientists did not express immunoreactive Vpu from the EcoRI-KpnI fragment
2 of ARV2 in 1984.

3 An ordinarily skilled scientist working in 1984 with a viral isolate which
4 contained full-length Vpu and Env would have expressed Env along with Tat, Rev, and
5 Vpu (Rekosh 1988, Bird 1990, Kimura 1994). Although it was unnecessary to the
6 recombinant Env immunoassay described in example 9 of the Chiron patent
7 application, if an ordinarily skilled scientist had chosen to perform additional analysis
8 on the product of the EcoRI-KpnI fragment expressed in COS cells, routine
9 electrophoretic mobility and immunoreactivity studies of the expressed proteins would
10 have both confirmed the presence of Env and distinguished Env from the unknown HIV
11 proteins (Tat, Rev, and Vpu). The size of Env as indicated on the sequence of ARV2
12 in the Chiron application combined with the art in 1984 taught an ordinarily skilled
13 scientist at the time that Env would be expressed as a large precursor and would be
14 processed into two large polypeptides. Whereas Env polypeptides measure 160, 120,
15 and 41 kDa, Tat, Rev and Vpu measure only 14, 19, and 16 kDa, respectively (Hauber
16 1987, Cohen 1988, Strebel 1988, Cullen 1988, Ruben 1989, Felber 1989, Hope 1990).
17 In October 1984, as predicted by the ARV2 sequence described in the Chiron
18 application, Tat, Rev, and Vpu would all have been demonstrably too small to be Env.

19 C. Plasmid vectors which could transcribe randomly generated short
20 DNA fragments of unknown nucleotide sequence in all three open reading frames were
21 available for the expression of HIV peptides in 1984 (Gray 1982). However, in
22 October 1984, without accurate sequence information of the fragments and of the
23 original viral genome an ordinarily skilled scientist would not have known from which
24 part of the virus or the host genome these fragments arose.

25 D. In 1984, to generate recombinant polypeptides from Env of any
26 HIV strain, an ordinarily skilled scientist would follow the guidelines of the Chiron
27 patent application. The application states that other isolates of HIV can be found in

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1 patients with AIDS or ARC. In 1984, methods for obtaining new isolates from lymph
2 nodes or blood of patients were well known in the art (Barre-Sinoussi 1983, Gallo
3 1984, Schupbach 1984, Levy 1984, Luciw 1984). Indeed, the application teaches that
4 two HIV isolates had been identified as of October 1984, one at the Institut Pasteur and
5 the other at the Laboratory of Tumor Cell Biology at The National Institutes of Health
6 (Barre-Sinoussi 1983, Popovic 1984). Two more viral isolates were characterized in
7 the application (ARV3 and ARV4) and their sequences were described as being
8 polymorphic. Thus, the Chiron patent application teaches that different isolates of HIV
9 will have variability in sequence and that more viral isolates should be obtained and
10 characterized.

11 Routine collaborations with hospitals serving communities in which the
12 AIDS virus had been observed, as reported in medical journals and in publications such
13 as Morbidity and Mortality Weekly Reports from the Center for Disease Control,
14 would have and did yield sufficient patients and tissues to identify many new strains of
15 HIV (Myers 1994). The Chiron application describes how to grow the virus in tissue
16 culture cells and how to clone the viral genome. The application teaches that DNA
17 hybridization will yield homologous sequences from new genomic or cDNA libraries.
18 It also directs the sequencing of the viral genome, which was routine in 1984.

19 All HIV1 and HIV2 *env* sequences are sufficiently similar to permit the
20 identification of their *env* ORFs with the HIV *env* probe described in the Chiron patent
21 application (Myers 1994). Moreover, the amount of genetic diversity among *env* genes
22 from various HIV1 and HIV2 isolates would not preclude an ordinarily skilled scientist
23 from extrapolating the Chiron disclosure regarding the location of *env* in ARV2 to all
24 other isolates of HIV1 and HIV2 (Clavel 1986, Guyader 1987, Myers 1994).

25 These steps have been followed since 1984, resulting in the rapid
26 accumulation of viral strains and sequences as well as the identification of HIV2 and
27 SIV (Clavel 1986, Hirsch 1986, Hahn 1987, Charneau 1994, Myers 1994). The

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1 genomic organization of these lentiviruses, which was first outlined in the Chiron
2 application, was confirmed. In HIV1 and HIV2, the location, size and features of *env*
3 are maintained as are the strategies of viral replication and gene expression (Sanchez-
4 Pescador 1985, Guyader 1987, Hirsch 1987). Therefore, an ordinarily skilled scientist
5 could have followed the teachings of the Chiron application together with the art of
6 1984 to collect a variety of additional HIV1 and HIV2 isolates, to clone them, to
7 sequence them, to identify and characterize their *env* genes, and to make and use
8 recombinant Env immunoassays.

9 E. Dr. Jäck asserts that an inordinate number of disparate peptides
10 from different strains of HIV would have to be examined to obtain smaller peptides to
11 use as immunoreactive reagents against Env. Dr. Jäck does not explain how this
12 assertion relates to the construction of a recombinant Env immunoassay in October
13 1984. If, for example, he means to suggest that his "hundreds of millions" of amino
14 acid sequences must be examined to find short peptides which are broadly cross-
15 reactive, he is wrong. In October 1984, an ordinarily skilled scientist would not have
16 needed to take the approach suggested by Dr. Jäck to determine broadly cross-reactive
17 short peptides. At the time, there were a number of well-known principles for
18 predicting the immunoreactivity of a peptide (Sutcliffe 1983, Singh 1984). For
19 example, instead of randomly expressing peptides from different strains of HIV, a
20 small number of viral isolates would be sequenced and compared.

21 The Chiron application teaches that the three viral isolates from
22 San Francisco were polymorphic, i.e., DNA fragments of different lengths were
23 generated following their digestion with various restriction endonucleases. Following
24 this observation, an ordinarily skilled scientist would have looked for differences in
25 protein sequence between viral isolates in 1984. Routine examination of different Env
26 sequences would have revealed hypervariable, variable, and conserved domains of the
27 protein (Veronese 1985, Starcich 1986, Modrow 1987, Alizon 1987, Myers 1994).

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1 Conserved sequences would then be examined for their hydrophilicity and
2 hydrophobicity profiles and possible secondary structures (Sutcliffe 1983, Singh 1984).
3 In the art of 1984, immunoreactive oligopeptides of predetermined lengths from
4 conserved sequences were known to interact with specific antibodies (Laver & Air
5 1980, Wiley 1981, Benjamin 1984). Such routine approaches were in fact followed
6 and yielded highly immunoreactive peptides from conserved regions of Env (Gnann
7 8/1987, Gnann 9/1987, Bugge 1990, Bottiger 1990, Krowka 1991, Sprengers 1991,
8 Broliden 1992).

9 Based upon the teachings of the Chiron application and what was known
10 in the art in October 1984, there was no need to screen hundreds of millions of variable
11 sequences. The application identified *env* ORF, demonstrated its expression in cells,
12 and demonstrated its polymorphism. Armed with this information, an ordinarily skilled
13 scientist in 1984 could isolate and characterize new viral strains as well as predict,
14 design and test the immunoreactive peptides from Env of HIV1 and HIV2.

15 F. It would not require extensive experimentation to express Env in
16 host cells not known in 1984.

17 The Chiron application does not require the use of any specific expression
18 system, or purport to claim the invention of expression systems. In October 1984,
19 scientists of ordinary skill were routinely expressing heterologous genes in a wide
20 variety of expression systems, including bacteria, plants, yeast, insect cells, and
21 mammalian cells (e.g., Smith 1983, DiNocera 1983, Chernajovsky 1984, Old &
22 Primrose 1985). After Chiron showed that Env expressed in mammalian cells was
23 immunoreactive with an AIDS serum, an ordinarily skilled scientist would easily have
24 combined the teachings of the patent with any of these expression systems known in
25 1984 or later to subclone the *env* fragment identified by the Chiron application into
26 appropriate vectors and determine levels and fidelities of expression of recombinant
27 proteins.

28

1 Moreover, Dr. Jäck is incorrect in stating that the expression systems he
2 lists were not known in 1984. Most expression systems in use today were either known
3 in October 1984, or are very similar in function to systems known in 1984. In fact,
4 Chiron scientists and others have expressed Env in a variety of host cells and viral
5 systems, including E. coli (Crowl 1985, Schneider 1987, Morikawa 1990), yeast (Barr
6 1987), baculovirus (Hu 1987, Morikawa 1990); CHO cells (Lasky 1986), COS cells
7 (Sanchez-Pescador 1985), vaccinia virus (Chakrabarti 1986), and Drosophila cells
8 (Ivey-Hoyle 1990). WOP cells and expression in sheep were not known in 1984.
9 However, WOP cells represent a system analogous to COS cells (Kern & Basilico
10 1986, Ward 1994), and moreover, neither WOP cells nor expression in sheep has
11 gained wide following in the field of gene expression.

12 G. In October 1984, a scientist of ordinary skill in the field at issue
13 had an advanced degree (a masters, Ph.D., or M.D.) and experience in at least one of
14 the three relevant technical areas: virology, immunology, or molecular biology, as
15 well as some knowledge of immunoassays.

16 H. I have reviewed the opinions expressed by Dr. John Young in his
17 report of May 1, 1995, and I agree with the conclusions expressed therein and the bases
18 therefor.

19 III. COMPENSATION AND PRIOR TESTIMONY

20 I have not testified as an expert at trial or by deposition within the last
21 four years. My compensation in connection with this matter is \$270 per hour.

22 IV. INFORMATION AND REFERENCES CONSIDERED

23 In addition to Dr. Jäck's report and the references listed therein, and my
24 experience in working in the field, as reflected in the publications listed in my
25 curriculum vitae, I considered the content of the following list of articles and I have
26 consulted with Dr. Young regarding the materials set out in Dr. Young's report at
27 Section IV in forming my opinions. To exemplify the basis for the opinions set forth

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1 above, I have referred to certain of these references in the text. These textual
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